“Endophytic Plant Growth Promoting Bacteria from Turmeric for Enhancing yield Of Turmeric Rhizome

A synopsis submitted to

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Introduction

Turmeric *Curcuma longa,* a widely recognized medicinal plant, has been valued for centuries due to its broad therapeutic applications, largely attributed to its bioactive compounds, including curcumin. Traditionally used in Ayurvedic and traditional Chinese medicine, turmeric has been recognized for its anti- inflammatory, antimicrobial, and antioxidant properties. Recent studies have turned attention to the plant's endophytic community, focusing on endophytic bacteria that reside within the plant tissues without causing harm, and play significant roles in plant health and growth.

Endophytic bacteria, often regarded as plant growth-promoting bacteria (PGPB), exhibit remarkable abilities that benefit their host plants. These bacteria contribute to enhanced nutrient uptake, stimulate growth through phytohormone production, induce resistance to pathogens, and improve plant resilience to environmental stresses. Specifically, in medicinal plants like turmeric, endophytic bacteria may also contribute bioactive secondary metabolites that complement or even enhance the medicinal properties of the host. Thus, isolating and characterizing endophytic bacteria from turmeric presents an opportunity to explore novel bacterial strains with unique bioactivities and plant growth-promoting traits.

India, as a primary producer of turmeric, offers a rich diversity of plant-associated microorganisms due to its varied agro-climatic zones. However, research on turmeric's endophytes, especially bacterial communities, remains relatively underexplored. This research proposes to isolate and characterize endophytic bacteria from turmeric, evaluating their plant growth-promoting properties and potential biotechnological applications. The ultimate aim is to identify bacterial strains that can act as biofertilizers or biocontrol agents, promoting sustainable agriculture by reducing reliance on chemical fertilizers and pesticides.

Turmeric *Curcuma longa*, a perennial herbaceous plant of the Zingiberaceae family, is renowned for its extensive use in traditional medicine, particularly in South Asia. Its therapeutic properties, attributed largely to its bioactive compound curcumin, include antimicrobial, antioxidant, antiinflammatory, and anticancer activities. Beyond these phytocheEmicals, recent studies have uncovered the significant role of endophytes— microorganisms living symbiotically within plant tissues—in contributing to the plant's bioactivity. These endophytes have gained attention as a novel source of bioactive metabolites, with promising applications in agriculture, pharmaceuticals, and biotechnology.

The pioneering work of Bacon and White (2000) laid the foundation for understanding plantendophyte interactions, suggesting that endophytes not only enhance plant resilience to biotic and abiotic stress but also produce secondary metabolites with pharmaceutical potential. Similarly, Strobel et al. (2004) demonstrated that endophytes from medicinal plants could serve as reservoirs of unique bioactive compounds. This has driven a surge in research exploring endophytic diversity and their bioprospecting potential.

Despite turmeric's established medicinal reputation, its bacterial endophytes remain largely unexplored. Prior studies by Pandey et al. (2018) and Verma et al. (2019) revealed the existence of diverse bacterial communities in medicinal plants, emphasizing their role in enhancing plant health and producing antimicrobial agents. However, comprehensive studies isolating and characterizing endophytes from *Curcuma longa* are limited. Addressing this gap, this study aims to isolate, characterize, and identify bacterial endophytes from turmeric plants to explore their potential bioactive properties

Objectives

- To isolate and identify Bacterial endophytes from turmeric
- To study Morphological Biochemical and Molecular Characterization of bacterial isolate
- To screen the isolated endophytes for various bio activities
- To conduct pot assay

Review of literature

- **Ajay Kumar, et al (2016)**

isolate bacterial strain from rhizome of curcuma longa were characterised on the basis of morphology and biochemical characteristics and 16 s rRNA sequencing Isolates were identified to six strains namely *Bacillus cereus,Bacillus thuringenesis,,Bacillus pumilis , Pseudomonas putida*

- **Sulistiyani Sulistiyani & Tri Ardyati, Sri Winarsi(2016)**

Most cases of bacterial resistance towards antibiotics, encourage various efforts to gain new sources of antibiotics. Endophyte bacteria is a microorganism has important role as the producer of bioactive compounds. Endophyte bacteria from Curcuma longa with antimicrobial and antioxidant activities h not been studied yet. *Curcuma longa*has been utilized as the main ingredients of traditional herbal medicines (*jamu*). The objective of this research was to investigate the antimicrobial and antioxidant activity of endophyte bacteria associated with *Curcuma longa* rhizome. Based on morphological characteristics of bacterial colonies, eight endophyte bacteria was isolated from *Curcuma longa* rhizome. Screening of endophyte isolate has antimicrobial activity was done using agar well diffusion method.

- **Verinder Virk, Himani Deepak & Khushbu Taneja (2014).**

Curcuma longa L., popularly known as Haldi; member of Zingiberaceae family, is one of the most popular and useful herbal medicinal plant. The tremendous research proven that most of the medicinal properties of turmeric are due to the curcumin content in it. Turmeric has other various useful properties such as treatment of wounds, menstrual pain relief, antioxidants, anti-inflammatory, anticancerous, antiulcer, etc. Turmeric healthy rhizomes and roots are continuously in close association with the rhizospheric region and as a result, large number of rhizospheric-associated beneficial microbes interacts with the underground parts of plants. Soil microbes may neutralize the action of flavonoids released from the roots and get entry into the healthy tissues of plants to become endophytes. Rhizospheric and endophytic species either directly or indirectly involved in propounding beneficial impact to the plant. The present review emphasized in order to uncover the mutualistic association of turmeric and its associated microbes. Also the natural products from medicinal plant associated microbes have pharmacological, biological and therapeutic values which might favour mankind in future for drug designing and novel drug formulation

- **Garima Sharma et al (2024)**

Endophytes contribute to plant fitness and defence by producing bioactive secondary metabolites, which may be utilized in various industrial applications. We assessed the potential of bioactive compounds from the endophytes of *Curcuma longa* for therapeutic properties. We screened phytochemicals obtained from the *C. longa* endophytes for host-specific bioactive compounds which were also evaluated for their antimicrobial, anti-inflammatory and antioxidant potential.

- **G Vinayarani & HS5985648 Prakash (2018)**

Plant growth promoting rhizobacteria and endophytic bacteria were isolated from different varieties of turmeric (Curcuma longa L.) from South India. Totally 50 strains representing, 30 PGPR and 20 endophytic bacteria were identified based on biochemical assays and 16S rDNA sequence analysis. The isolates were screened for antagonistic activity against Pythium aphanidermatum (Edson) Fitzp., and Rhizoctonia solani Kuhn.,

causing rhizome rot and leaf blight diseases in turmeric, by dual culture and liquid culture assays. Results revealed that only five isolates of PGPR and four endophytic bacteria showed more than 70% suppression of test pathogens in both assays. The SEM studies of interaction zone showed significant ultrastructural changes of the hyphae like shriveling, breakage and desication of the pathogens by PGPR B. cereus (RBac-DOB-S24) and endophyte P. aeruginosa (BacDOB-E19). Selected isolates showed multiple Plant growth promoting traits. The rhizome bacterization followed by soil application of B. cereus (RBacDOB-S24) showed lowest Percent Disease Incidence (PDI) of rhizome rot and leaf blight, 16.4% and 15.5% respectively. Similarly, P. aeruginosa (BacDOB-E19) recorded PDI of rhizome rot (17.5%) and leaf blight (17.7%). The treatment of these promising isolates exhibited significant increase in plant height and fresh rhizome yield/plant in comparison with untreated control under greenhouse condition. Thereby, these isolates can be exploited as a potential biocontrol agent for suppressing rhizome rot and leaf blight diseases in turmeric.

- **Alain-Martial Sontsa-Donhoung et al (2022)**

ial endophytes may enhance plant growth and stress tolerance. Yet, the plant health benefits of endophytes can be altered by biotic and abiotic factors and, thus, favour the inhibition of turmeric growth and curcumin production. The double petri dish method and greenhouse pot experiments were conducted to assess the biocontrol potential and impact of endophytes on the output, curcumin levels, and antioxidant activities of turmeric (*Curcuma longa*L.). The results showed that endophytes could control some disease-causing plant pathogens: 52% of all isolates have an antagonistic action against *Fusarium oxysporum*, 43% against *Pythium myriotylum*, 35% against *Phytophthora megakarya*, and 56% against *Ralstonia solanacearum in vitro*. Eight months after sowing, most endophyte isolates can increase the yield of turmeric rhizomes on a sterile substrate after inoculation, with yields ranging from 42 to 105% higher than the control and 3 to 50% higher than the urea treatment. In addition, 52% endophytes isolate significantly raised curcumin levels after 8 months of culture (from 2.1 to 3.1%) compared to control (1.7%) and urea treatment (1.8%). These endophytes promote an increase in the levels of reduced glutathione (22%), total thiols (26%), and carotenoids (91%) in turmeric. The study concludes that, in general, the endophytes-turmeric association can stimulate turmeric rhizome production, curcumin, and the antioxidant activities of the plant. They can also be used as biocontrol agents for plant pathogens.

- **Shaju Reema& Thankam, Suba GA Manuel (2023)**

The endophytes are non-pathogenic microorganisms that colonize the internal parts of the plant, helping in enhancing the plant growth mechanism and protection by helping the host plants to produce phytohormones like Indole Acetic Acid, gibberellins and cytokinin which promotes the growth of the plant. They elicit the plant in producing secondary metabolites that keeps the pests and insects at bay and has antimicrobial properties against plant pathogens, thus protecting the host during abiotic and biotic stress conditions. Most of the herbal plants are in association with microorganisms which help in producing compounds with medicinal value. *Curcuma longa* (turmeric) is a medicinal plant that has been traditionally used for thousands of years to treat diseases. The underground turmeric rhizome favors the growth of various microbial communities which synthesizes secondary metabolites which modulates the plant growth. In the present study, bacterial endophytes were isolated from *C. longa*. Their ability to enhance Plant Growth-Promoting activities (nitrogen, IAA, phosphate, zinc, HCN) and the resistance toward abiotic (salt), biotic stresses were assessed against certain bacterial and fungal pathogens (antimicrobial properties). Six of the endophytic culture isolates showed PGP activity which were selected and identified using 16S rRNA sequencing technique. The isolates were identified as *Kocuria rocea,* (MT317205.1), *Bacillus subtilis* (MT068199.1), *Brevibacterium casei* (GQ365205.1) *Actinobacterium JS14 strain*, (AY372899.1), *Bacillus Amyloliquefaciens*(MT131178.1) and *Bacillus velezensis*(CP028204.1). The PGP activities of endophytes were seen to improve the performance of the host plants and its resistance against biotic and abiotic stresses and provides protection against plant pathogen.

Methodology 1.Collection of turmeric sample

✞ **Plant selection:**Select healthy turmeric plants from different growing reagions ✞
Sampling: Collect healthy turmeric rhizomes to isolate endophytic bacteria

2.Isolation of endophytic bacteria

✞ **Sterilization of plant tissue:**

- the collected plant rhizomes by rinsing them under the running water.
- Then surface sterilize them by soaking in 70% of ethanol for 30 seconds followed by 5%sodiumhypochloride solution for 3 to 5 minutes
- .Finally rinse thoroughly with sterile distilled water to remove any surface contaminants ✞ **Bacterial isolation:**
- Cut the sterilized plant rhizomes in small segments
- Plate the tissue surface on nutrient media(Nutrient agar or tryptic soya agar TSA)
- Incubate at 28C temp for 48to 72 hours for bacterial growth
- The isolated bacteria from the tissue surfaces sub cultured to obtain pure colonies,

3.Identification of endophytic bacteria

✞ **Morphological identification**
Observe the colony morphology and perform gram staining
✞ **Biochemical testing** catalase, oxidase, and motility to characterize bacterial isolates.
✞ **Molecular Identification**
16S rRNA gene sequencing to identify the bacterial species.

4. Screening of isolates for PGPB traits such as:

- Nitrogen fixation (e.g., Ashby's Mannitol medium)

- Phosphate solubilization (using Pikovskaya's agar medium)

- Production of plant growth hormones (IAA By using Salkowskis method)

- Antagonistic activity against pathogen- (using dual culture techniques)

Production of sidrophore*(Chrome azurols method)

5.Inoculation of Turmeric Plants with PGPB

Preperation of inoculum: Grow selected endophytic PGPB Strains in liquid medium(e.g. nutrient broth

Inoculation: For root inoculation soaked the turmeric rhizome in the bacterial suspension before planting

Control group: Include the control group without any bacterial inoculation to compare the effect of PGPB **6.**

Data collection and statistical analysis

8.Growth parameters: Regularly monitor and record data on plant height,leaf number Rhizome

size and weight

Importance of study (society applications) International status

Importance of Studying Endophytic Bacteria from Turmeric Across Various Countries

1. **China**

- focuses on bioactive compounds from endophytes in medicinal plants, including turmeric, for pharmaceutical uses. Chinese research in traditional medicine often explores endophytes for bioactive secondary metabolites, contributing to potential drug innovations.
- The methodologies and compound screenings developed in China offer valuable protocols and insights for countries interested in pharmacology and biotechnological applications of endophytes.

2. **United States**

- The U.S. focuses on both agricultural and medicinal aspects, often incorporating advanced molecular tools for identifying and characterizing endophytes.
- U.S. research advances the scientific understanding of endophytes at the molecular level and supports the development of bio-based products for sustainable agriculture, which can inform policies and practices worldwide.

3. **Germany and Other European Countrie**s

- European countries like Germany emphasize regulatory frameworks and safety assessments for using endophytes as biocontrol agents and biofertilizers.
- Insights from Europe are valuable in establishing international standards and safety protocols for applying endophytes in agriculture, ensuring safe and effective usage.

4. **Southeast Asian Countries** (e.g., Thailand, Indonesia):

- These countries focus on endophytes from medicinal plants for agriculture and traditional medicine enhancement.
- Insights from Southeast Asia provide valuable knowledge about the symbiotic relationships between native plants and endophytes in tropical environments, benefiting sustainable agricultural practices and biodiversity conservation efforts globally.

Studying the international research on turmeric endophytes enhances knowledge-sharing across borders, strengthens agricultural and medicinal applications, and contributes to global biodiversity and conservation efforts. Insights from various countries enable a comprehensive approach to utilizing these beneficial microorganisms for sustainable development worldwide.

National status

Research on Endophytic Bacteria from Turmeric in Different Cities of India

1. **Pune (Maharashtra)**

- Pune hosts several prominent research institutions, including the National Chemical Laboratory (NCL) and the University of Pune, where studies focus on the biotechnological and industrial potential of turmeric endophytes.
- Research in Pune often explores the production of enzymes, bioactive compounds, and biofertilizers derived from turmeric endophytes, contributing to agricultural and pharmaceutical applications.

2. **Bengaluru (Karnataka)**

- Bengaluru's Indian Institute of Science (IISc) and University of Agricultural Sciences (UAS) are centers for advanced research on microbial diversity, focusing on turmeric's endophytic bacteria for applications in biofertilizers and biocontrol.
- The city's biotechnology industry is also exploring commercial applications of these endophytes, aiming to develop sustainable agricultural products.

3. **New Delhi**

- Research institutions in New Delhi, such as the Indian Agricultural Research Institute (IARI) and the Department of Biotechnology (DBT), are actively studying turmeric endophytes for their role in sustainable agriculture and soil health improvement.
- Government-funded projects focus on using turmeric endophytes to enhance crop yield and resilience to pests and diseases, with a strong emphasis on translating research into practical applications for Indian farmers.

4. **Hyderabad (Telangana)**

- In Hyderabad, institutions like the Centre for Cellular and Molecular Biology (CCMB) and the National Institute of Nutrition (NIN) focus on understanding the genetic and biochemical properties of turmeric endophytes.
- Research in Hyderabad emphasizes the bioactive potential of endophytes for health applications, contributing to drug discovery and the development of natural health supplements.

5. **Lucknow (Uttar Pradesh)**

- The Central Institute of Medicinal and Aromatic Plants (CIMAP) in Lucknow is a leader in studying the medicinal properties of endophytes from turmeric and other aromatic plants.
- Lucknow's research efforts include isolating endophytic bacteria with potential antimicrobial and antioxidant activities, with the goal of enhancing the medicinal value of turmeric in herbal medicine and Ayurveda

In summary, research on turmeric endophytes is actively conducted in various Indian cities, each contributing to different aspects—agricultural, medicinal, and ecological. This city-specific research reflects India's diverse approach to utilizing turmeric endophytes for sustainable agriculture, biotechnology, and health innovations across varied climates and applications.

Significance

The isolation of bacterial endophytes from turmeric plants holds significant importance due to their diverse applications in agriculture, medicine, and biotechnology. These endophytes, residing within plant tissues without causing harm, play a crucial role in enhancing plant health and productivity. They promote plant growth by producing phytohormones such as indole-3-acetic acid (IAA), solubilizing essential nutrients like phosphorus, and fixing atmospheric nitrogen, thus improving nutrient uptake. Additionally, endophytes enhance the plant's resistance to biotic and abiotic stresses by producing antimicrobial compounds, siderophores, and enzymes that degrade harmful pathogens. This makes them a valuable tool in developing eco-friendly biocontrol strategies, reducing dependence on chemical fertilizers and pesticides. In turmeric, a plant known for its medicinal properties, these endophytes may contribute to its pharmacological activity by synthesizing bioactive metabolites with antibacterial, antifungal, and antiinflammatory properties. The study of these bacteria also provides deeper insights into plant-microbe interactions and their role in enhancing turmeric's medicinal and therapeutic potential. Furthermore, isolated endophytes can be explored for novel bioactive compounds with applications in drug discovery, agriculture, and environmental sustainability, emphasizing their broad scientific and practical relevance.

Formulation and Structure of Study:

Year-wise Plan of work and targets to be achieved

Sr.no	Year	Activity	Tentative Duration
1)	First Year	• Literature Review and Methodology Setup • Sample Collection • Isolation and Culturing of Endophytic Bacteria Preliminary Identification (Morphological & Biochemical Tests)	12 Months
2)	Second Year	• Molecular Identification • Bioactivity Screening	12 Months
3)	Third Year	• Pot assay • Summary • Conclusion • Documentation • Analysis of result • Submission of thesis	12 Months

Presentation of the research work, attending conferences, workshops will be done alongside all the activities as per mentioned in above table.

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